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**RATE AND EFFECTS OF PCB  
ACCUMULATION ON *EISENIA FOETIDA*  
FINAL REPORT  
30 MAY 1989-30 SEPTEMBER 1989**

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**ABSTRACT**

The purpose of this final report is to supplement earlier toxicity tests exposing *Eisenia foetida* to Aroclor 1254 PCB. The data provided in this report are from three related studies: relationship of PCB exposure to reproductive success, rate of PCB bioaccumulation by *Eisenia* in artificial soil and rate of PCB bioaccumulation by *Eisenia* placed in naturally-occurring PCB contaminated soils. Conclusions reached from the results of these experiments will be used to enhance the predictive capabilities of bioassay and field bioassessment procedures that are currently being applied to dredged material and contaminated soils.



## MATERIAL AND METHODS

*Eisenia foetida* was grown in a medium composed of fresh horse manure and peat moss. Cultures were kept at 20°C and at 90% humidity [8]. Feeding of fresh horse manure was on weekly basis. Test worms were hand sorted for size (300-600 mg) and visual sexual maturity (clitellum present).

The PCB used for testing was Aroclor 1254, technical grade. Diluted stocks were prepared from the concentrated Aroclor liquid and added to the test soil in 0, 10 and 100 mg/kg on a dry soil basis. Each aliquot of PCB liquid was weighed to the nearest 0.1 mg before being diluted to a 250 ml volume with Pesticide-grade acetone.

Artificial soil was prepared according to standard EEC Ecotoxicity testing guidelines [5]. Each test vessel contained 500 mg dry of a mixture of fine sand (70%), kaoline clay (20%) and fine peat moss (10%). The pH of this mixture was adjusted to between 5.5 and 6.0 using CaCO<sub>3</sub>. The pH of the artificial soil was not changed after the addition of the PCB [1]. The amount of artificial soil needed for each test was mixed by batch in an electric cement mixer, sieved through a 6 mm sieve and sealed in a plastic bag for overnight at 4°C. The overnight storage allowed moisture equilibration throughout the media. After 24 hours, the soil moisture content was measured and adjusted as necessary to 35% water by weight.

A 100 g portion of dry sand per container was withheld from the artificial soil ingredients for mixing with the PCB. This sand was spread evenly in a 20 cm round petri dish and 25 ml of the PCB stock solution added. The PCB/acetone solution saturated the entire volume of sand and was allowed to dry for 2 hours under a fume hood. After the 2 hours drying, the petri dish was covered with glass to reduce any PCB volatilization. A hand-held electric mixer was used to mix in the PCB/sand mixture with the artificial soil.

In addition to the PCB, each container also received 10 g of dry, finely ground cow manure as a food source [6]. Distilled water was added to obtain a final moisture level of 55% on a dry weight basis. The test medium was then transferred from the stainless steel mixing bowl to a glass test pot. Lids were used to reduce evaporation from the moist artificial soil mixture during the test. Each test pot received 5 clitellated earthworms weighing between 300 and 600 mg fresh weight. Worms were hand sorted from the stock culture and then randomly divided between all test pots. The fresh weight of each 5 worms/pot was



recorded and the total weight of each pot with worms checked weekly to maintain the correct moisture level.

At the completion of a test, the worms were hand sorted from the test material, counted and weighed. The artificial soil from which the worms had been removed was then sieved for cocoons using a 14 mm stainless steel sieve. Soil samples for analysis, pH and moisture content were taken before sieving for cocoons.

Cocoon sieving was performed in a closed water system so that the water and soil could be disposed of properly. The sieved cocoons were rinsed, blotted dry and weighed as groups to the nearest 0.1 mg. They were then placed in containers separating each cocoon so that hatching success might be monitored. The temperature for the cocoon hatching was 20°C. They were observed 3 times weekly for new hatchlings. After hatchlings were counted, they were discarded.

Earthworm tissue samples for chemical analysis were cleaned, rinsed, blotted and weighed directly after sorting from the test medium. Then they were purged overnight on moist filter paper at 20°C. The filter paper was renewed after 2, 18 and 36 hours purging. At 48 hours the worms were again cleaned and weighed as before. They were placed in acetone rinsed glassware and stored at -20°C for analysis.

The soil and tissue samples were analysed for 11 PCB congeners, DDE and Aroclor 1254. The PCBs were removed from a sample of approximately 6 g of soil with the aid of steam distillation at pH 3. In the course of the distillation, the distillate was extracted with hexane [2,3]. The extract was purified [4] by passing it over a column of silica-gel (Baker 7086-3 or Bond Elut Si 601303) mounted on a column of benzenesulphonic acid (Baker 7090-3 or Bond Elut SCK 617303). The extract was then analysed for PCBs with the aid of gas chromatography using electron capture detection.

A sample of approximately 4 g of worms was subjected to proteolysis with the aid of pepsin. The PCBs were then removed from the homogeneous product by steam distillation at pH 3. In the course of the distillation, the distillate was further extracted with hexane [2,3]. The extract was further treated as the soil samples.

PCB congener structures were assigned on basis of retention times. The determinations were carried out with the aid of methods involving external standards. The standards were subjected to the same procedures as the test samples.



Statistical procedures were used to evaluate the data. The analysis of variance and Waller-Duncan k-ratio test were used to determine if statistical differences existed and to identify those differences. Both procedures were conducted at the 0.05 level of significance [10].

## RESULTS

### Artificial soil uptake study

In keeping with earlier exposure tests, no earthworm mortality was measured at the 10 or 100 ppm treatments (Table 1). Earthworm weights increased significantly from Day 0 to Day 56 for all treatments (Table 2). During this period, the 10 ppm treatment had a 37% and the 100 ppm treatment a 32% increase in weight compared with a 47% for the control.

Earthworm reproduction also increased significantly during the 56-Day exposure to the PCB-contaminated artificial soil (Table 3). Cocoon numbers increased consistently 2-3 times at all sample dates for the 100-ppm treatment and numbered approximately 2 times the control at Day 56. Earthworms in the 10-ppm treatment showed their greatest increase (2.8 x) in cocoon production at Day 28 with no significant increase at Day 56. This treatment was 22% greater than the control at Day 56.

*Eisenia* tissue concentrations of Aroclor 1254 were 3-4 times greater than artificial soil concentrations (Figures 1, 2). Soil analysis of contaminated artificial soil showed 50-60% of the nominal concentration. Also, soil analysis of Day 0 and Day 56 samples (n = 1) showed no noticeable difference for the artificial soils or the naturally-occurring soils.

A comparison of Aroclor 1254 tissue uptake rates during the 56-day test for the 10 and 100 ppm exposure showed no statistical difference from Day 7 to Day 56 except for a decrease at Day 28 for the 10 ppm test (Table 4, 5). PCB congener uptake in tissues was generally similar to that of Aroclor 1254 although several congeners showed statistically significant increases in tissue concentrations measured at Day 7 and 14 compared with Day 56 (Figure 3). All congener tissue concentrations for the 10 ppm test decreased at Day 28 as for the Aroclor. Converse to the Aroclor uptake during the 100 ppm test, a large number of congeners' uptake decreased at Days 28 and 56 compared with Days 7 and 14 (Table 5). Selected congeners from the 100 ppm treatment with varying uptake responses over time are shown in Figure 4.



### Reproduction versus PCB concentration

Cocoons were harvested after 14 and 28 days exposure to 0, 32, 56, 100, 180, 320 and 560 ppm nominal PCB Aroclor 1254 (Tables 6, 7). At Day 14, cocoons were harvested for all treatments except 180, 320 and 560 ppm. For Day 28, cocoons were harvested for all treatments except 320 and 560 ppm. For both sample dates the largest number of cocoons were harvested from worms exposed to the 100 ppm PCB and the smallest from the 32 ppm PCB.

The per cent cocoons hatching was significantly greater for Day 14 cocoons than Day 28 (75-100% vs. 38-75%) respectively (Tables 6, 7). The average number of worms hatched per cocoon for Day 14 ranged from 2.4 for the 100 ppm treatment to 3.33 for the 32 ppm exposure. The average number hatched per cocoon for Day 28 ranged from 2.0 (180 ppm) to 3.0 (56 ppm). Average cocoon weights for both sample dates ranged from 0.013-0.015 g, wet.

The number of days necessary for cocoon hatching varied only slightly for treatments and time. The earliest hatchings were measured at day 34 and the latest at day 78. Although statistical comparisons were not made, the Day 28 cocoons from the 180 ppm exposure appeared to be more delayed than the other treatments. The time for a cocoon to complete the hatching process (the first to the last worm hatched) ranged from 1-14 days, with the average being 5-8 days.

### Naturally contaminated soils

*Eisenia* exposed to the naturally-occurring PCB contaminated soils burrowed and survived in two of the three soils. The worms exposed to soil # 2 were removed after three days when it became apparent that they were not burrowing in the soil. An oily film was observed on their skin at this time. When placed on wet filter paper for purging, they did not purge any soil.

Worms removed from soil # 1 after the 28 day exposure appeared thinner and slower moving than those from the control and soil # 3. The per cent weight reduction for the control worms was  $27\% \pm 2.4$ ,  $40\% \pm 1.3$  for soil # 1 and  $30\% \pm 1.3$  for soil # 3 (Table 8). Cocoons were observed after 28 days in the clean soil but not in either of the contaminated soils.



Tissue concentrations of Aroclor 1254 from worms exposed to soil # 1 and soil # 3 were 5-6 times greater than the soil (Figure 5). In addition, tissue concentrations of Aroclor 1254 in the worms exposed to soil # 1 were approximately 3 times that of the worms exposed to 10 ppm artificial soil even though the analytically measured Aroclor 1254 in each was nearly the same, 5.8 ppm vs. 6.2 ppm, respectively (Table 9 and Figure 6). PCB congener concentrations in tissue exposed to the contaminated soils were significantly above background and 1.4 times greater for soil # 1 than # 3 (Table 10).



## DISCUSSION

The time-related effects of exposing *Eisenia foetida* for 56 days to artificial soil contaminated with Aroclor 1254 PCB were measured. The weight of the earthworms exposed to 10 and 100 ppm Aroclor 1254 made a significant increase from Day 0 to Day 56 except for slight weight decreases at Day 14 and 28 for the 10 ppm and 100 ppm treatments respectively. Cocoon numbers likewise increased in time with a significantly greater increase measured for the 100 ppm exposure than for the 10 ppm or control treatments.

Earthworm bioconcentration factors were 3-4 for Aroclor 1254 in this 56 Day exposure to contaminated artificial soil. These comparisons were made on a wet/wet basis. Other studies [4] have shown 5-6 times greater earthworm tissue levels (approximately 600 ppm/wet) compared with soil levels (approximately 130 ppm/dry). Considering the 35% moisture level of the artificial soil used in this test, an approximate comparison could be made to these wet/dry values by dividing the wet/wet values of this report by 1.5.

There were marked differences in the uptake rate of Aroclor 1254 as well as many individual PCB congeners for the 10 and 100 ppm treatments.

Similarly, tissue samples from both treatments were statistically equal to their maximum Aroclor 1254 level at Day 56. But the 100 ppm treatment reached this maximum level at Day 7 and remained there throughout the test; whereas for the 10 ppm treatment showed a more gradual increase in tissue concentrations for Days 7 and 14 and a decrease for Day 28. PCB congener uptake for the 10 ppm treatment was similar to the Aroclor with a very pronounced drop in levels at Day 28 but an increase again at Day 56. PCB congener uptake for the 100 ppm treatment was similar to that of the Aroclor with maximum uptake at Days 7 and 14, but many Day 28 congener levels decreased. At Day 56, the only congeners not decreasing significantly from Day 7 and 14 levels were the higher chlorinated ones.

The apparent reason for the reduction in PCB congener levels at Days 28 and 56 for the 100 ppm treatment would seem to be due to the earthworms ability to excrete lower chlorinated PCBs but not the higher ones [4]. This would explain why three of the higher chlorinated congeners remained high throughout the test. For the lower level 10 ppm treatment, the slower uptake rate may have permitted the worms to begin excreting the PCBs earlier (Day 28) than at the higher concentration. However, it appears that the worms may once again increase their tissue levels even after an excretion process has begun, which may signal long-term inability to excrete even lower chlorinated PCBs.

There was a significant decrease in hatching success for cocoons harvested at Day 28 than at Day 14. This decrease from a minimum hatching success at Day 14 of 75% to a minimum success of 37% for Day 28 is similar to other findings [1] where 17-53% hatching success during exposure tests was recorded compared with 80% hatching under controlled conditions.

Measurements of cocoon weights did not correlate with the number of hatchlings from each cocoon. Also, a delay in cocoon production and hatching was seen for the highest PCB treatment (180 ppm), for which cocoons were produced. This data are in agreement with similar results by others [9].

The PCB uptake by one of the contaminated soils was significantly greater than that of the 10 ppm artificial soil treatment even though the PCB Aroclor 1254 measured analytically was very close for both. This may indicate that the artificial soil test may underestimate the actual uptake potential of an earthworm in relation to naturally-occurring contaminated soils. The analytically measured PCB concentrations for Day 0, 28 and 56 were approximately the same. This constancy was probably due to the fact that most PCBs in soil do not volatilize rapidly or succumb to microbial degradation [7].

A comparison of relative abundance of PCB congeners found in soil to those found in tissue showed approximately the same relationship between congeners for the 10 and 100 ppm treatments. Similar comparison of congeners from soil samples representing naturally-contaminated soils and their exposed tissues revealed a decrease in some congeners and an increase in others.

This study demonstrated the feasibility of using an artificial soil medium to measure the sublethal effects of PCB exposure to *Eisenia foetida* with time. Further studies would be helpful in understanding these effects and their impact on earthworm survival.

## **ACKNOWLEDGEMENTS**

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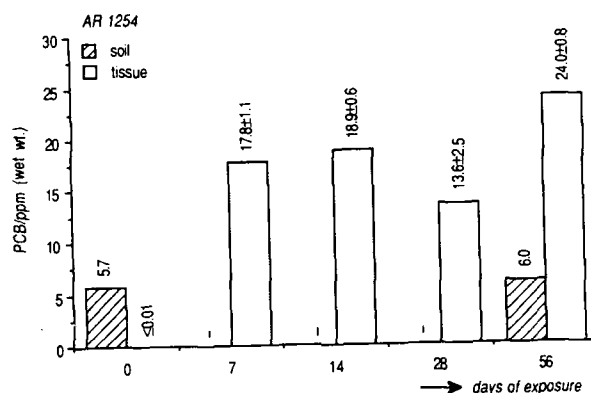
## REFERENCES

- [1] Bouwman, H. and A.J. Reinecke, 1987  
Effects of Carbofuran on the earthworm *Eisenia foetida* using a defined medium  
Bull. Environ. Contam. Toxicol. 38: 171-178.
- [2] Congener percentage in polychlorinated biphenyl (PCB)  
DIN 51-527, German Standard (draft) 1986.
- [3] Determination of polychlorinated biphenyls (PCBs) in mussel  
R 84/147, TNO, Delft, 1984.
- [4] Diercxsens, D. de Weck, N. Borsinger, B. Rosset and I. Tarradellas, 1985  
Earthworm contamination by PCBs and heavy metals  
Chemosphere 14: 511-522.
- [5] E.E.C. (European Economic Community),  
Directive 79/831, Annex V,  
Part C: Methods for the determination of ecotoxicity - level 1, earthworms artificial  
soil  
Comm. Euro. Communities, DG XI/128/82, Rev. 5, 1984.
- [6] Heimbach, F., 1984  
Correlations between three methods for determining the toxicity of chemicals to  
earthworms  
Pestic. Sci. 15: 605-611.
- [7] Pal, D., J.B. Weber and M.R. Overcash, 1980  
Fate of polychlorinated biphenyls in soil plant systems  
Residue Rev. 74: 45-98.
- [8] Reinecke, A.J. and J.R. Kriel, 1980  
The influence of temperature on the reproduction of the earthworm *Eisenia foetida*  
(Oligochaeta)  
SA J. Zool 16: 96-100.

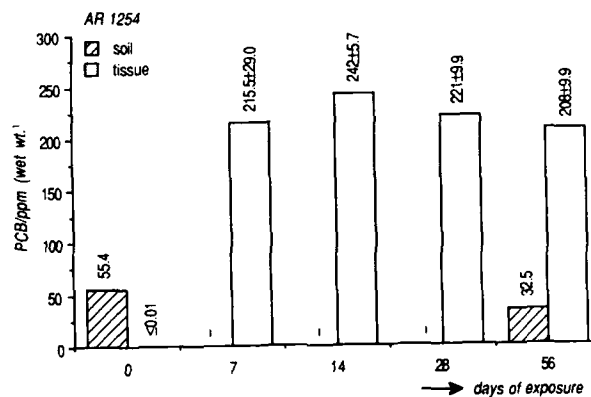


- [9] Reinecke, A.J. and J.M. Venter, 1985  
Influence of dieldrin on the reproduction of the earthworm *Eisenia foetida*  
(Oligochaeta)  
Biol. Fert. Soils 1: 39-44.
- [10] Steel, R.G.D. and J.H. Torrie, 1980  
Principles and procedures of statistics  
2nd ed. McGraw-Hill Book Co., New York.

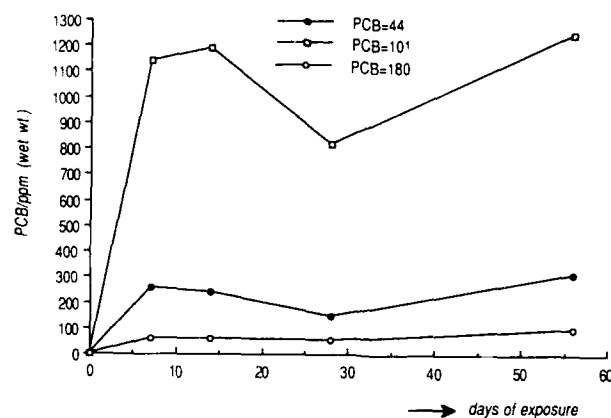




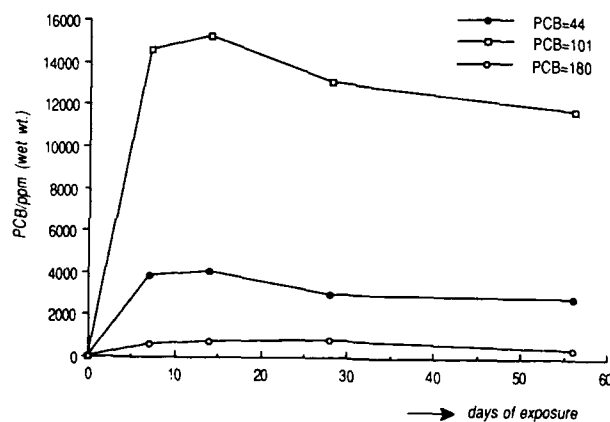
**Figure 1** Soil and tissue concentrations of Aroclor 1254 after 56-Day exposure to 10 ppm Aroclor 1254 in artificial soil.



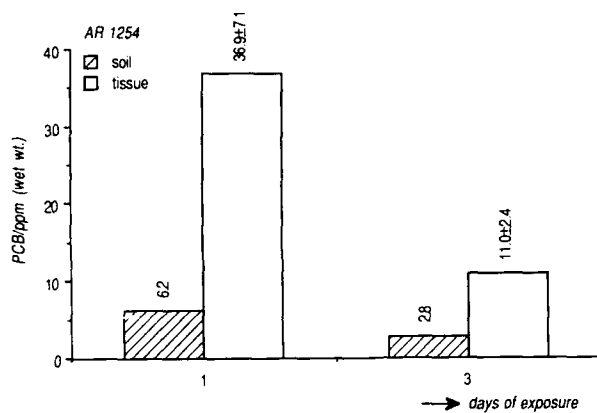
**Figure 2** Soil and tissue concentrations of Aroclor 1254 after 56-Day exposure to 100 ppm Aroclor 1254 in artificial soil.



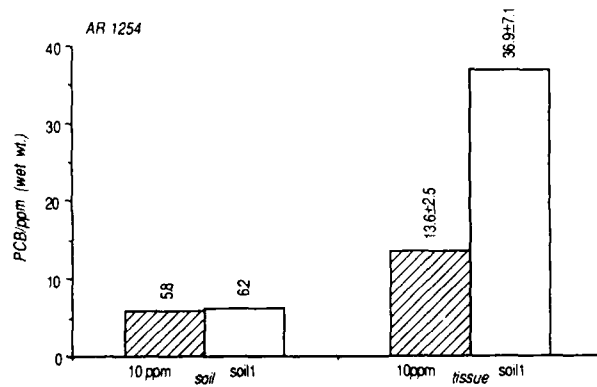
**Figure 3** Selected PCB congener concentrations from earthworm tissue after 56 day exposure to 10 ppm Aroclor 1254 in artificial soil.



**Figure 4** Selected PCB congener concentrations from earthworm tissue after 56 day exposure to 100 ppm Aroclor 1254 in artificial soil.



**Figure 5** Soil and tissue concentrations of Aroclor 1254 after 28 day exposure to two naturally-occurring contaminated soils.



**Figure 6** Soil and tissue concentrations of Aroclor 1254 from 28 day to 10 ppm Aroclor 1254 in artificial soil and a naturally-occurring contaminated soil.



**Table 1** *Earthworm weights and cocoon numbers for 56-Day Exposure to PCB-contaminated artificial soil.*

Sample I.D.	Worms wet weight (g)**/Cocoon no.				
	Day 0	Day 7	Day 14	Day 28	Day 56
0-A*	2.139	2.536 (4)	3.060 (4)	3.121 (16)	3.252 (15)
0-B	2.460	2.871 (5)	2.683 (8)	2.935 (11)	3.080 (19)
0-C	2.210	2.610 (1)	2.561 (5)	2.880 ( 8)	3.143 (12)
0-D	1.957	2.255 (5)	2.383 (8)	2.667 ( 5)	3.350 (22)
10-A	2.455	2.821 (3)	2.772 (7)	2.629 (19)	3.232 (28)
10-B	2.384	2.757 (3)	2.398 (7)	2.098 (25)	2.997 (18)
10-C	2.232	2.838 (3)	2.870 (8)	2.131 (11)	3.328 (23)
10-D	2.151	2.605 (5)	2.822 (4)	2.714 (18)	3.071 (24)
100-A	2.039	2.246 (3)	2.229 (5)	2.268 (15)	2.801 (34)
100-B	2.331	2.449 (2)	2.535 (4)	2.339 (15)	3.035 (27)
100-C	2.230	2.654 (2)	2.625 (4)	2.410 ( 6)	2.713 (40)
100-D	2.210	2.446 (1)	2.172 (5)	2.492 (14)	3.075 (32)

\* 0 = control treatment

10 = 10 ppm Aroclor 1254 treatment

100 = 100 ppm Aroclor 1254 treatment

\*\* 5 worms/container - 100% survival for all treatments.

**Table 2** *Analysis of variance of earthworm weights measured during 56-Day exposure to PCB-contaminated artificial soil.*

Sample I.D.	Mean worm wet weight (g)**				
	Day 0	Day 7	Day 14	Day 28	Day 56
0	2.19 ± 0.21 D**	2.57 ± 0.25 C	2.67 ± 0.29 BC	2.90 ± 0.19 AB	3.21 ± 0.12 A
10	2.31 ± 0.14 C	2.76 ± 0.11 B	2.72 ± 0.22 B	2.39 ± 0.32 C	3.16 ± 0.15 A
100	2.20 ± 0.12 C	2.45 ± 0.17 B	2.39 ± 0.22 BC	2.38 ± 0.10 BC	2.91 ± 0.18 A

\* 0 = control treatment

10 = 10 ppm Aroclor 1254 treatment

100 = 100 ppm Aroclor 1254 treatment

\*\* Different letters indicate statistical difference at  $p \leq 0.05$ .

**Table 3** Analysis of variance for cocoons produced by earthworms exposed to PCB contaminated artificial soil for 56 days.

Sample I.D.	Mean cocoon nos.			
	Day 7	Day 14	Day 28	Day 56
0	3.8 ± 1.9 C**	6.8 ± 1.5 BC	10.0 ± 4.7 B	17.0 ± 4.4 A
10	3.5 ± 1.0 B	6.5 ± 1.7 B	18.3 ± 5.7 A	20.8 ± 3.2 A
100	2.0 ± 0.8 C	4.5 ± 0.6 C	12.5 ± 4.4 B	33.3 ± 5.4

\* 0 = control treatment

10 = 10 ppm Aroclor 1254 treatment

100 = 100 ppm Aroclor 1254 treatment

\*\* Different letters indicate statistical difference at  $p \leq 0.05$ .



**Table 4** Analysis of variance of tissue samples exposed to 10 ppm Aroclor 1254 for days 0, 7, 14, 28, 56.

PCB congener	Day 0	Day 7	Day 14	Day 28	Day 56
PCB-15*	21.5 ± 4.9 A†	29.5 ± 0.7 A	34.5 ± 3.5 A	31.0 ± 14.1 A	34.5 ± 9.2 A
PCB-28	7.5 ± 0.8 D	39.0 ± 1.4 B	39.0 ± 1.4 B	27.5 ± 4.9 C	50.0 ± 1.4 A
PCB-52	14.0 ± 2.8 C	739.5 ± 26.2 A	699.5 ± 31.8 A	461.5 ± 68.6 B	754.5 ± 7.8 A
PCB-49	3.7 ± 0.6 D	174.5 ± 6.4 AB	158.0 ± 0.0 B	92.0 ± 11.3 C	178.0 ± 7.1 A
PCB-44	—	260.0 ± 7.1 B	242.0 ± 4.2 B	147.0 ± 25.5 C	314.0 ± 17.0 A
PCB-70	5.1 ± 1.3 D	485.0 ± 4.2 B	486.5 ± 0.7 B	287.0 ± 35.4 C	550.0 ± 32.5 A
PCB-101	15.5 ± 4.9 C	1139.5 ± 34.6 A	1191.0 ± 55.2 A	819.5 ± 119.5 B	1245.5 ± 16.3 A
PCB-87	4.6 ± 1.6 C	549.0 ± 35.4 A	567.0 ± 0.0 A	337.5 ± 41.7 B	617.5 ± 40.3 A
PP-DDE	4.4 ± 1.1 D	190.5 ± 6.4 B	205.0 ± 1.4 AB	126.5 ± 16.3 C	232.0 ± 19.8 A
PCB-153	15.5 ± 3.5 D	539.0 ± 58.0 BC	588.5 ± 23.3 B	461.5 ± 74.2 C	820.0 ± 14.1 A
PCB-138	14.0 ± 4.2 D	700.5 ± 55.9 BC	791.0 ± 29.7 B	608.5 ± 94.0 C	1171.0 ± 35.4 A
PCB-180	6.9 ± 1.6 C	60.5 ± 7.8 B	59.0 ± 4.2 B	53.5 ± 12.0 B	99.0 ± 4.2 A
AR 1254**	—	17.8 ± 1.1 AB	18.9 ± 0.6 AB	13.6 ± 2.5 B	23.5 ± 0.8 A

\* PCB congeners concentrations in µg/kg (ppb) wet weight.

\*\* Aroclor 1254 concentration in mg/kg (ppm) wet weight.

† Different letters indicate statistical difference at  $p \leq 0.05$ .

— Below detection.

**Table 5** Analysis of variance of tissue samples exposed to 100 ppm Aroclor 1254 for days 0, 7, 14, 28, 56.

PCB congener	Day 0	Day 7	Day 14	Day 28	Day 56
PCB-15*	21.5 ± 4.9 D†	697.5 ± 91.2 A	546.0 ± 9.9 AB	398.5 ± 38.9 BC	316.5 ± 65.8 C
PCB-28	7.5 ± 0.8 D	515.0 ± 50.9 AB	523.5 ± 26.9 A	411.0 ± 11.3 BC	330.5 ± 62.9 C
PCB-52	14.0 ± 2.8 C	10820.0 ± 1371.8 A	10355.0 ± 176.8 A	8322.5 ± 173.2 B	7237.5 ± 194.5 B
PCB-49	3.7 ± 0.6 D	2422.5 ± 251.0 A	2460.0 ± 63.6 A	1902.5 ± 53.0 AB	1690.0 ± 7.1 BC
PCB-44	—	3827.5 ± 357.1 A	4017.5 ± 272.2 A	2904.0 ± 49.5 B	2830.0 ± 113.1 B
PCB-70	5.1 ± 1.3 C	6320.0 ± 572.8 A	6645.0 ± 318.2 A	5222.5 ± 173.2 B	4827.5 ± 180.3 B
PCB-101	15.5 ± 4.9 D	14572.5 ± 2124.9 A	15225.0 ± 438.4 A	13087.5 ± 307.6 AB	11752.5 ± 979.3 BC
PCB-87	4.6 ± 1.6 E	7085.0 ± 671.8 AB	7760.0 ± 162.6 A	6397.5 ± 187.4 BC	5705.0 ± 219.2 CD
PP-DDE	4.4 ± 1.1 D	2387.5 ± 378.2 ABC	2872.5 ± 95.5 A	2432.5 ± 38.9 AB	2167.5 ± 67.2 BC
PCB-153	15.5 ± 3.5 B	6482.5 ± 788.4 A	7505.0 ± 7.1 A	7630.0 ± 360.6 A	7415.0 ± 360.6 A
PCB-138	14.0 ± 4.2 C	8065.0 ± 648.5 B	9487.5 ± 152.0 A	9840.0 ± 480.1 A	9480.0 ± 282.8 A
PCB-180	6.9 ± 1.6 D	615.0 ± 66.5 C	748.0 ± 4.2 B	811.0 ± 66.5 AB	888.5 ± 6.4 A
AR 1254**	—	215.5 ± 29.0 A	242.0 ± 5.6 A	221.0 ± 9.9 A	208.0 ± 9.9 A

\* PCB congeners concentrations in µg/kg (ppb) wet weight.

\*\* Aroclor 1254 concentration in mg/kg (ppm) wet weight.

† Different letters indicate statistical difference at  $p \leq 0.05$ .

— Below detection.

**Table 6** Cocoon hatching for *Eisenia foetida* exposed to Aroclor 1254 for 14 days.

Treatment PCB (ppm)	Total no. of cocoons	% Cocoons hatched	Average weight per cocoon (wet, g)	Average no. worms per cocoon	Days to* hatch
0	11	100	0.015	2.73 ± 0.9	34-55
32	7	86	0.015	3.33 ± 0.52	34-48
56	13	100	0.013	2.77 ± 1.17	34-52
100	20	75	0.013	2.40 ± 1.12	34-57
180	None				
320	None				
560	None				

\* Minimum/maximum days = day 0 hatching = day 0 exposure.

**Table 7** Cocoon hatching for *Eisenia foetida* exposed to Aroclor 1254 for 28 days.

Treatment PCB (ppm)	Total no. of cocoons	% Cocoons hatched	Average weight per cocoon (wet, g)	Average no. worms per cocoon	Days to* hatch
0	35	60	0.015	2.57 ± 1.16	45-69
32	31	43	0.014	2.31 ± 1.38	38-69
56	38	37	0.014	3.0 ± 1.18	50-69
100	45	38	0.015	2.76 ± 1.15	44-69
180	12	75	0.013	2.0 ± 1.12	41-78
320	None				
560	None				

\* Minimum/maximum days = day 0 hatching = day 0 exposure



**Table 8** Earthworm weights, mortality and reproduction after 28 day exposure to naturally-occurring contaminated soil.

Sample* I.D.	Worms wet weight (g)/live worms**		Cocoons
	Day 0	Day 28	
0-A	2.035	1.460	Yes
0-B	2.761	1.939	Yes
0-C	2.335	1.771	Yes
0-D	2.909	2.108	Yes
1-A	2.654	1.284/4	No
1-B	2.481	1.536	No
1-C	2.486	1.488	No
1-D	2.183	1.298	No
3-A	1.895	1.323	No
3-B	2.694	1.897	No
3-C	2.540	1.798	No
3-D	2.231	1.514	No

\* 0 = control

1 = soil # 1

3 = soil # 3

\*\* N = 5 unless otherwise noted.

**Table 9** Earthworm PCB tissue concentrations exposed to naturally-occurring PCB contaminated soil and artificial soil contaminated to 10 ppm and 100 ppm Aroclor 1254.

	Contaminated soil	10 ppm	100 ppm
PCB-15*	42.5 ± 13.4 B †	31.0 ± 14.1 B	398.5 ± 38.9 A
PCB-28	29.0 ± 1.4 B	27.5 ± 4.9 B	411.0 ± 11.3 A
PCB-52	438.0 ± 35.4 B	461.5 ± 68.6 B	8322.5 ± 173.2 A
PCB-49	99.5 ± 0.7 A	92.0 ± 11.3 A	1902.5 ± 53.0 A
PCB-44	220.0 ± 21.2 B	147.0 ± 25.5 B	2940.0 ± 49.5 A
PCB-70	184.0 ± 17.0 B	287.0 ± 35.4 B	5222.5 ± 173.2 A
PCB-101	1249.5 ± 159.1 B	819.5 ± 119.5 B	13087.5 ± 307.6 A
PCB-87	368.0 ± 65.1 B	332.5 ± 41.7 B	6397.5 ± 187.4 A
PP-DDE	320.5 ± 44.5 B	126.5 ± 16.3 C	2432.5 ± 38.9 A
PCB-153	2126.0 ± 335.2 B	461.5 ± 74.2 C	7630.0 ± 360.6 A
PCB-138	1920.0 ± 349.3 B	608.5 ± 94.0 C	9840.0 ± 480.8 A
PCB-180	894.5 ± 263.8 A	53.5 ± 12.0 A	811.0 ± 66.5 A
AR 1254**	36.9 ± 7.1 B	13.6 ± 2.5 C	221.0 ± 9.9 A

\* PCB congeners concentrations in µg/kg (ppb) wet weight.

\*\* Aroclor 1254 concentration in mg/kg (ppm) wet weight.

† Different letters indicate statistical difference at  $p \leq 0.05$ .



**Table 10** Analysis of variance for tissue samples exposed to two PCB contaminated soils for 28 days.

PCB congener	Day 0	Soil 1	Soil 3
PCB-15*	21.5 ± 4.9 B †	30.0 ± 1.4 AB	42.5 ± 13.4 A
PCB-28	7.5 ± 0.8 C	22.0 ± 2.8 B	29.0 ± 1.4 A
PCB-52	14.0 ± 2.8 C	203.5 ± 23.3 B	428.0 ± 35.3 A
PCB-49	3.7 ± 0.6 C	55.5 ± 7.8 AB	59.5 ± 0.7 A
PCB-44	- - C	111.5 ± 13.4 B	220.0 ± 21.2 A
PCB-70	5.1 ± 1.3 C	88.0 ± 39.6 AB	184.0 ± 17.0 A
PCB-101	15.5 ± 4.9 C	552.5 ± 99.7 B	1249.5 ± 159.1 A
PCB-87	4.6 ± 1.6 B	233.5 ± 82.7 A	368.0 ± 65.1 A
PP-DDE	4.4 ± 1.1 C	96.0 ± 18.4 AB	320.5 ± 44.5 A
PCB-153	15.5 ± 3.5 C	525.5 ± 98.3 AB	2126.0 ± 335.2 A
PCB-138	14.0 ± 4.2 C	592.0 ± 144.2 B	1920.0 ± 349.3 A
PCB-180	6.9 ± 1.6 C	104.5 ± 0.7 AB	894.5 ± 263.8 A
AR 1254**	- - B	11.0 ± 2.4 A	86.9 ± 7.1 A

\* PCB congeners concentrations in µg/kg (ppb) wet weight.

\*\* Aroclor 1254 concentration in mg/kg (ppm) wet weight.

† Different letters indicate statistical difference at  $p \leq 0.05$ .

- Below detection.